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FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
28911/36128	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	
09/485245	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB98/02550	21 August 1998	22 August 1997

TITLE OF INVENTION
LABELLING COMPOSITION AND METHOD

APPLICANT(S) FOR DO/EO/US
HOPKINS, Alison

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
   b ☐ have been transmitted by the International Bureau.
   c ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4))
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/485245	PCT/GB98/02550	28911/36128

21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1 482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**	
☒	International preliminary examination fee (37 CFR 1 482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1 482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	6 - 20 =	0	x $18.00	**$0.00**
Independent claims	1 - 3 =	0	x $78.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**
	TOTAL OF ABOVE CALCULATIONS =			**$970.00**
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1 9, 1.27, 1.28) **(check if applicable).**			☐	**$0.00**
	SUBTOTAL =			**$970.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1 492 (f)).			+	**$0.00**
	TOTAL NATIONAL FEE =			**$970.00**
Fee for recording the enclosed assignment (37 CFR 1 21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**
	TOTAL FEES ENCLOSED =			**$970.00**
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$970.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **13-2855** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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31,879
REGISTRATION NUMBER

07 February 2000
DATE

PATENT APPLICATION

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

Applicant:	"EXPRESS MAIL" mailing label No. EM362733790US
Alison Hopkins	
	Date of Deposit: February 7, 2000
U.S. National Phase (35 USC 371) Application Based on PCT/GB98/02550 filed 21 August 1998	I hereby certify that this paper (or fee) is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.
Serial No.: To Be Determined	
Filed: Herewith	
For: Labelling Composition and Method	
Group Art Unit: To Be Determined	
Examiner: To Be Determined	Richard Zimmermann

PRELIMINARY AMENDMENT ACCOMPANYING FILING
OF NATIONAL STAGE APPLICATION UNDER 35 U.S.C. 371

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

AMENDMENT

In the Claims:

Please amend claims 3, 4 and 5 as follows:

--3. [AMENDED] A labelling composition as claimed in claim 1 [or claim 2], wherein the random mixture is of 6-mer oligonucleotides.--

--4. [AMENDED] A labelling composition as claimed in claim 1 [any one of claims 1 to 3], wherein the composition is present in a freeze-dried state.--

--5. [AMENDED] A method of making labelled probes for a nucleic acid template, which method comprises incubating the nucleic acid template under chain extension conditions with the labelling composition of claim 1 [any one of claims 1 to 4].--

REMARKS

The foregoing amendments are made to eliminate various multiple dependencies and to place the claims in condition for allowance. An early notice of allowance is hereby solicited. Should the Examiner wish to discuss any matter of form or substance, he or she is invited to contact the undersigned attorney at the number listed below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By _____
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February 7, 2000

LABELLING COMPOSITION AND METHOD

This invention concerns compositions comprising random mixtures of oligonucleotides and their use for labelling nucleic acids by a random prime method.

Feinberg and Vogelstein (1, 2) introduced the use of random sequence hexanucleotides to prime DNA synthesis on denatured template DNA at numerous sites along its length. The primer-template complex is a substrate for the "Klenow" fragment of DNA polymerase I. By replacing a non-radioactive nucleotide with the radiolabelled equivalent in the reaction mixture, newly synthesised DNA is made radioactive.

Very small amounts of input DNA can be labelled, enabling very high specific activity probes to be produced with relatively small quantities of added nucleotides. These radioactive labelled fragments can then be used as sensitive hybridisation probes for a wide range of filter based applications (3-6).

There are several labelling kits that are commercially available for the labelling of DNA by the random prime method. These include the Multiprime, Megaprime, Rediprime and Fluorescein Gene Images kits available from Amersham International plc. Ready-To-Go kits are available from Pharmacia and High Prime kits are available from Boehringer.

The Multiprime kit was introduced in the 1980s. It provides different tubes containing the different solutions that enable the user to make up labelling mixtures. One such tube contains a random mixture of 6-mer oligonucleotides, another the polymerase enzyme, and another the supply of nucleotides in the reaction buffer. All these separate solutions are stored frozen at -20°C. The purchaser thaws the different solutions, and adds precise quantities of each to the sample of denatured DNA that is

to be labelled, including a labelled nucleotide. This reaction is then usually incubated at 37°C at which temperature, oligonucleotide annealing and chain extension can occur. However, the reaction may also be incubated at lower temperatures such as an ambient room temperature of about 20°C.

The Megaprime kit was introduced commercially in the early 1990s. It is similar to the Multiprime kit, except that 9-mer oligonucleotides are used in place of 6-mers. The Megaprime kit has an advantage over the Multiprime kit, in that 9-mer oligonucleotides anneal more strongly (than do 6-mers) to a DNA target and form a hybrid having a higher melting temperature. Thus 9-mers achieve better and more rapid priming of a target then do 6-mers.

The Rediprime kit was introduced commercially in 1994. It comprises a mixture of 9-mer oligonucleotides with a polymerase enzyme and a supply of nucleotides. The mixture is supplied in a freeze-dried state. The freeze-dried mixture also contains a dye for easy visualisation. Dried kits for performing nucleic acid manipulation experiments were described by Ortlepp and McKay in EP 298 669 entitled "Performing nucleic acid reactions". The user reconstitutes the mixture by adding liquid containing the DNA template that is to be labelled, and then liquid containing the labelled nucleotide.

The Ready-To-Go kit was introduced during the 1990s. It is based on a random prime solution containing a random mixture of 9-mer or longer oligonucleotides, which solution is dried by a technique described in EP 383 569. A dye is not present. Like the Rediprime kit, the Ready-To-Go kit can be stored at +4°C or at ambient temperature. Promotional literature emphasises the speed of labelling, which results from the use of 9-mer oligonucleotides.

The High Prime kit is a wet kit containing a random mixture of oligonucleotides. The kit literature does not indicate what length of random oligonucleotides are used, but in the related document EP 649 909 A2, the

use of 6-mer, 9-mer, 12-mer and 15-mer is disclosed. No preferred length of random oligonucleotide is given. The solution is stablised by the use of glycerol and can be stored at between about -20°C and +4°C.

It can be seen that there has been a trend in commercial kits towards the use of longer oligonucleotides, particularly 9-mers or even longer. Going against this trend, it has been determined by Suganuma, A and Gupta, K C (7) that the use of long random primers, especially 9-mers or longer, reduces the priming efficiency of the random primer reaction. These authors worked on solutions which were used without being dried at any stage. The conclusions of these authors conflict with the findings of the present inventors; which findings are to the effect that, when experiments are done with solutions which are not dried, 9-mers provide more rapid and efficient labelling than do 6-mers, and do not give rise to any problem resulting from self-annealing or self-priming. To the best of applicants' knowledge, the conclusions reported by the authors of (7) have not caused the suppliers of random prime kits to use shorter oligonucleotides.

The present invention is based on the discovery that self-annealing occurs when random 9-mers are used in dried predispensed labelling kits, and that this limits their stability and shelf life. The self-annealing occurs during dispensing and storage when the random 9-mers anneal together to form primer-dimers or primer concatemers. These primer complexes become labelled during the normal labelling reaction, which concomitantly reduces the amount of label that is incorporated into copies of the template that are being synthesised during the reaction. Shorter oligonucleotides are not subject to this problem. The problem is specific to 9-mers (and longer oligonucleotides) used in dried kits.

The invention provides a labelling composition comprising a random mixture of oligonucleotides which are 6-mers to 8-mers, said composition present in a dry state. Preferably the composition also contains at least one of: a polymerase enzyme; a supply of nucleotides for

chain extension; a labelled nucleotide; a dye; a stabiliser; and a buffer.

As the experimental data below shows, 5-mer oligonucleotides are too short to be useful in dried kits. As the length of the oligonucleotides increases from 6-mers to 9-mers, there is a concomitant increase in the aforementioned self-priming problem. On the other hand, longer oligonucleotides anneal more rapidly and strongly to templates than do shorter ones. Taking into account both these factors, applicants believe that 6-mer oligonucleotides are more preferable than 7-mers which in turn are more preferable than 8-mers.

The random mixture of oligonucleotides is present in a dry state. Various drying techniques are possible, including that described in EP 383 569, and also freeze-drying or lyophilisation which is preferred.

It is possible to use any DNA polymerase enzyme in the labelling reaction, for example Klenow, exonuclease free klenow, DNA polymerase I, T7 DNA polymerase, Sequenase™, Thermosequenase™, so long as the reaction buffer conditions are suitable for the specific enzyme being used.

All four of the nucleotides are preferably present in the composition, whether labelled or unlabelled, and the relative molar concentrations may be adjusted to improve the efficiency of labelling. Also when a labelled nucleotide is present, the equivalent unlabelled nucleotide may also be present to improve the efficiency of labelling, or to control the specific activity of the DNA that is being produced from the labelling reaction.

These compositions enable a DNA template to be used to produce copies which are labelled radioactively, for example, by using either phosphate labelled with P-32 or S-35, or by using H-3 or C-14 base labelled nucleotides. Alternatively non-radioactive labels may be used, for example, fluorescein, biotin, digoxigenin, rhodamine and cyanine dyes, may be incorporated when, for example, covalently linked to the base moiety of the nucleotide.

Any stabiliser may be present to protect the activity of the enzyme, for example, trehalose, sucrose, BSA, gelatin. A dye may also be present to allow the dried pellet to be visualised, before use, and to assist in determining that mixing is thorough.

The invention also includes a method of making labelled probes for a nucleic acid template, which method comprises incubating the nucleic acid template under chain extension conditions with the labelling composition as herein described. Preferably the template is DNA. The inventor has found that random 6-mers can give fast labelling kinetics (10 minutes labelling time) by being present at high concentration in the reaction mixture. A preferred concentration is 2-10 O.D./ml in the final reaction with about 5 O.D./ml being most preferable. A probe labelled in this manner is suitable for use in a Southern hybridisation.

All the results shown in the examples show labelling with dCTP-^{32}P, but this is only as a means to show, and quantitate the amount of self-priming that occurred in each reaction. The reactions are able to label DNA with other labels, both radioactive and non-radioactive, as indicated elsewhere in this specification.

References

1. Feinberg, A P and Vogelstein, B, Anal. Biochem., 132: 6-13 (1983).

2. Feinberg, A P and Vogelstein, B, Addendum Anal. Biochem., 137: 266-267 (1984).

3. Southern, E M, J. Mol. Biol., 98: 503-517 (1975).

4. Thomas, P S, Proc. Nat. Acad. Sci., USA, 77: 5201-5205 (1980).

5. Meinkoth, J and Wahl, G, Anal. Biochem, 138: 267-284 (1984).

6. Grunstein, M and Hogness, D S, Proc. Natl. Acad. Sci, USA, 72: 3961-3965 (1975).

7. Sugunuma, A and Gupta, K C, Analytical Biochemistry, 224: 605-608 (1995).

Example 1. Manufacture of lyophilised reactions with different random primer lengths:

All primers were diluted to 50 O.D./ml in water. The number of enzyme units was the same in each reaction (7 units).

The amount of each component solution is as follows for a 6 ml scale.

	5 mer reaction mix	6 mer reaction mix	7 mer reaction mix	8 mer reaction mix	9 mer reaction mix
Nucleotide buffer	1.998 ml	1.998 ml	1.998 ml	1.998 ml	1.998 ml
Exo-free Klenow (12 µl) 100 units/µl	1200 units	1200 units	1200 units	1200 units	1200 units
Dilution Buffer	28 µl	28 µl	28 µl	28 µl	28 µl
5 mer primer	1.0 ml				
6 mer primer		1.0 ml			
7 mer primer			1.0 ml		
8 mer primer				1.0 ml	
9 mer primer					1.0 ml
20% Trehalose	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml
0.2 mg/ml Xylene Cyanol	0.198 ml	0.198 ml	0.198 ml	0.198 ml	0.198 ml
PF Water	1.264 ml	1.264 ml	1.264 ml	1.264 ml	1.264 ml
Total Volume	6 ml	6 ml	6 ml	6 ml	6 ml

Each reaction mix was dispensed into tubes in 35 µl aliquots, and were freeze dried.

Methods:

1. Nucleotide buffer: Labelling buffer from Nick Translation kit (N5000/N5500 Amersham International plc).

2. Dilution buffer: Storage buffer for enzyme dilution.

3. Labelling Method: Tubes of DNA for labelling were made up as follows:

5 μl λ HindIII DNA at 5 ng/μl in TE buffer.

40 μl water.

Placed all tubes in a boiling water bath (95 to 100°C) for 5 minutes,

placed all tubes on ice for 5 minutes, centrifuged briefly,

then added the denatured DNA solutions to the respective dried reaction tube samples

added 5 μl Redivue™ dCTP ($\alpha^{32}P$) (Product Code AA0005: Amersham International plc) (50 μl total reaction volume).

Incubated all reactions for 10 minutes at 37°C.

Spotted 2 μl samples out onto PEI-cellulose tlc plates,

Ran plates in 1.25 M KH_2PO_4 pH 3.4.

Analysed plates on plate scanner, to measure the %incorporation, %self-priming and %dCTP present at the end of each reaction.

The %self-priming is defined as the % of the total radioactive counts that are situated between the incorporated counts and the counts due to the unincorporated dCTP-^{32}P.

λ HindIII DNA Labelling with dCTP-^{32}P (Week 1 Test)

Tube	Primer Type	Tube-1			Tube-2		
		% Incorp	% Self-Prime	% dCTP	% Incorp	% Self-Prime	% dCTP
1, 2	5 mers	62.7	7.9	23.0	54.8	7.7	30.9
3, 4	6 mers	79.9	11.2	2.7	82.1	10.8	2.2
5, 6	7 mers	73.5	17.5	2.8	74.3	15.1	3.7
7, 8	8 mers	68.3	19.1	3.2	65.6	20.4	3.6
9, 10	9 mers	64.9	23.7	3.1	61.5	27.2	3.1

The column headed "% Incorp" shows the percentage of dCTP-^{32}P incorporated as a chain extension product of a primer-λHind III DNA hybrid. The column headed "% Self-Prime" shows the percentage of dCTP-^{32}P incorporated in a complex involving only primers. The column headed "% dCTP" shows the percent of unincorporated dCTP-^{32}P. The % dCTP figures were unacceptably high when 5-mer oligonucleotides were used, but were acceptable for 6-mers to 9-mers. Within this range, the % Incorp figures decrease as the oligonucleotide length increases from 6 to 9.

Example 2. Long term stability comparison of dried reactions, nonamers compared with hexamers, 3.5 units of Exo-free Klenow per reaction:

The samples were made up as shown in Example 1, but 6 μl of Exo-free Klenow was used.

DNA Labelling with dCTP-^{32}P, results are the averages of the three reactions

	Nonamers			Hexamers		
Week	% Incorp	% Self-Prime	% dCTP	% Incorp	% Self-Prime	% dCTP
3	61.9	17.5	6.2	69.6	9.7	6.3
6	71.4	18.0	4.3	80.8	8.4	4.7
10	65.8	20.2	6.4	75.0	11.9	6.9
16	66.5	16.5	8.0	73.4	11.1	6.5
21	78.3	10.8	3.0	84.3	5.8	2.4
25	42.7	11.6	40.4	55.1	5.4	35.1

As these figures show, the % incorporation of dCTP-^{32}P when using 9-mers was initially lower than when using 6-mers and remained lower on storage of the compositions for up to 25 weeks.

Example 3:

Using dried reactions as shown in Example 1, the primer was replaced with water for the reaction drying, and was added later as a separate solution, when the reactions were being used. All reactions were incubated for 10 minutes, and then sampled to measure the % incorporation.

Primer Concentration in reaction O.D./ml	% Incorporation (hexamer primers) Average of two reactions	% Incorporation (nonamer primers) one reaction
6.0	78.3	
5.0	83.2	81.0
4.0	67.7	65.6
2.0	51.5	67.0
1.0	45.1	60.2

It can be seen from these results that the same primer concentration (O.D./ml) is required to achieve the same reaction kinetics, i.e. the same % incorporation in 10 minutes with different random primer lengths. This shows that the molar concentration needs to increase as the primer length is reduced.

Although the above results were obtained using wet reagents, the conclusion would apply also when dry primers are used.

Example 4:

Densitometer results of Southern hybridisations

25ng labelling reactions were carried out using the Megaprime Labelling Kit RPN 1606 (Amersham International plc) or using labelled probes from dried nonamer or hexamer labelling reactions made as described above in other examples. Southern blots were hybridised for 2 hours at 65°C with the labelled probe under standard conditions and then washed in 2 x SSC, 0.1% SDS, 20 minutes at room temperature, followed by two washes in 0.5 x SSC, 0.1% SDS, for 5 minutes 65°C. The dried blots were detected on X-ray film with 2 intensifying screens and place into a -70°C freezer, for 16 hours. After the film was developed using a film processor it was scanned using a densitometer, then the results were analysed using ImageQuant software.

Kit	Time of test after manufacture	Target	%band intensity of Southern hybridisation cf Megaprime control
9mers	1 week	0.25pg	42.23
9mers	1 week	0.5pg	40.12
9mers	1 week	1.0pg	38.93
6mers	1 week	0.25pg	97.09
6mers	1 week	0.5pg	95.02
6mers	1 week	1.0pg	94.33
6mers	37 weeks	0.25pg	74.58
6mers	37 weeks	0.5pg	80.91
6mers	37 weeks	1.0pg	81.17

Conclusions:

The hexamers used in a dried labelling reaction generate labelled probes which gave a much stronger band intensity than when nonamers are used, not only when tested initially after 1 week, but even after an extended period of storage (37 weeks at room temperature).

CLAIMS

1. A labelling composition comprising a random mixture of oligonucleotides which are 6-mers to 8-mers, said composition present in a dry state.

2. A labelling composition as claimed in claim 1, wherein the composition also contains at least one of: a polymerase enzyme; a supply of nucleotides for chain extension; a labelled nucleotide; a dye; a stabiliser; and a buffer.

3. A labelling composition as claimed in claim 1 or claim 2, wherein the random mixture is of 6-mer oligonucleotides.

4. A labelling composition as claimed in any one of claims 1 to 3, wherein the composition is present in a freeze-dried state.

5. A method of making labelled probes for a nucleic acid template, which method comprises incubating the nucleic acid template under chain extension conditions with the labelling composition of any one of claims 1 to 4.

6. A method as claimed in claim 5, wherein the random mixture of oligonucleotides is present at a concentration of 2-10 O.D./ml.

Atty. Docket No: 28911/36128

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ". LABELLING COMPOSITION AND METHOD ✓ _____," the specification of which (check one): ☐ is attached hereto; ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable); ☒ was filed as PCT International Application No. PCT/GB98/02550 ✓ on 21-08-1998 ✓ and was amended under Article 19 on _____ (if applicable). I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

			Priority Claimed	
9717972.5 ✓ (Application Serial Number)	GB ✓ (Country)	22-08-1997 ✓ (Day/Month/Year Filed)	☒ Yes	☐ No
(Application Serial Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)
(Application Serial Number)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
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Trevor B. Joike (25,542)
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APPENDIX

1. A labelling composition comprising a random mixture of oligonucleotides which are 6-mers to 8-mers, said composition present in a dry state.

2. A labelling composition as claimed in claim 1, wherein the composition also contains at least one of: a polymerase enzyme; a supply of nucleotides for chain extension; a labelled nucleotide; a dye; a stabiliser; and a buffer.

3. A labelling composition as claimed in claim 1, wherein the random mixture is of 6-mer oligonucleotides.

4. A labelling composition as claimed in claim 1, wherein the composition is present in a freeze-dried state.

5. A method of making labelled probes for a nucleic acid template, which method comprises incubating the nucleic acid template under chain extension conditions with the labelling composition of claim 1.

6. A method as claimed in claim 5, wherein the random mixture of oligonucleotides is present at a concentration of 2-10 O.D./ml.